

# LigaTrap® Human IgM Purification Kit

## Product Instructions

### Introduction

LigaTrap® Human IgM Purification Resin is engineered to purify high quality human IgM antibodies from recombinant and monoclonal sources. Binding capacity for monoclonal human IgM is  $\geq 15\text{mg/ml}$  resin. The LigaTrap® Human IgM Purification Kit provides all the necessary reagents for fast, convenient micro-scale purification of human IgM in just 10 easy steps. Each kit contains 10 microspin columns prefilled with 0.1ml of LigaTrap® Human IgM Purification Resin, buffers, and collection tubes. Each spin column can be used, regenerated, and used up to 10 times with minimal loss in binding capacity. Kappa and Lambda IgM can be purified using this product. **Serum applications are not recommended with all LigaTrap® IgM Purification products, due to potential cross reactivity with other immunoglobulins.**

### Kit Contents

Part #	Item	Quantity
LT-143-MSC	<b>Microspin Columns</b> - centrifuge columns supplied with caps and plug. Each column contains 0.1 mL LigaTrap® Human IgM Purification Resin in PBS buffer with 0.05% sodium azide.	10
BU-131-FP	<b>LigaTrap Sample Diluent 2.0</b>	15 mL
BU-132-FP	<b>LigaTrap Equilibration/Wash Buffer 2.0</b>	250 mL
BU-133-FP	<b>LigaTrap IgM Elution Buffer</b>	125 mL
BU-124-FP	<b>LigaTrap Regeneration Buffer</b>	50 mL
BU-125-FP	<b>LigaTrap Neutralization Buffer</b>	15 mL
BU-126-FP	<b>LigaTrap Storage Buffer</b>	50 mL
PL-057	<b>2 mL Collection Tubes</b>	80

### Additional Materials Required

- Microcentrifuge set between 1000-3000 x g
- Vortex/Mixer
- Centrifuge tubes or container for sample preparations

## Antibody Purification Procedure

### Sample Prep

1. In a separate tube (not supplied in kit) add 320µl of sample matrix (i.e. hybridoma supernatant or cell culture fluid) containing human IgM.
2. Add 80µl of **LigaTrap Sample Diluent 2.0** to the sample. Mix briefly by vortexing.

### Purification

3. Equilibrate resin by adding 400µl of **LigaTrap Equilibration/Wash Buffer 2.0**. Snap the bottom plug on the microspin column. Save the plug, as it will be needed to stopper the column. Insert the Microspin column into a supplied 2.0 mL Collection Tube. Centrifuge between 1000-3000 x g for 1 minute. Empty the buffer from Collection Tube. Repeat with two additional 400µl equilibrations. Insert the bottom plug into the microspin column.
4. Transfer 400µl of prepared sample (Step # 2) to the equilibrated column. Place screw cap on snugly. Vortex briefly for 15 seconds. Continue to mix/shake the sample and resin continuously for **5 minutes**.
5. Remove plug and insert the microspin column into a Collection Tube. Centrifuge between 1000-3000 x g for 1 minute. Discard unbound material or retain for further evaluation.
6. Add 400µl of the **LigaTrap Equilibration/Wash Buffer 2.0**. Remove plug and insert microspin column into a clean collection tube. Mix/shake resin continuously for 5 minutes. Centrifuge between 1000-3000 x g for 1 minute. Discard wash. Repeat with three additional washes for a total of four washes. (4x400µl)
7. Insert the washed microspin column into a **new, labeled** collection tube. Add 400µl of **LigaTrap IgM Elution Buffer** to the column. Vortex briefly for 15 seconds. Mix/shake resin continuously for 5 minutes. Centrifuge between 1000-3000 x g for 1 minute. Place the microspin column into a new, labeled Collection Tube. Repeat with second 400µl elution. ***\*Note: The eluates contain the purified antibodies. Do not discard!***
8. Pool eluates from Step 7 and add 80µl (10-12% v/v of elution samples) of **LigaTrap Neutralization Buffer** to the antibody. Attach the cap. Vortex briefly. The antibody will be near neutral pH. The antibody is ready for downstream applications. ***Note: There are no preservatives in the antibody. Use the antibody within one week or aliquot and store at -20° C or colder. Avoid multiple freeze thaws.***
9. If the column will not be reused, it can be discarded. If column is to be reused, regenerate the column by adding 400µl of **LigaTrap Regeneration Buffer**. Vortex briefly for 15 seconds. Mix/shake resin continuously for 5 minutes. Insert the Microspin column into a 2.0 mL collection tube and centrifuge between 1000-3000 x g for 1 minute.
10. To store resin, add 400µl of **LigaTrap Storage Buffer**. Insert the Microspin column into a 2.0 mL collection tube. Centrifuge between 1000-3000 x g for 1 minute. Discard wash. Repeat with an additional two washes for a total of three washes. (3x400µl). Insert the bottom plug into the Microspin column, add 400µl of fresh **LigaTrap Storage Buffer** and store at 2-8° C.